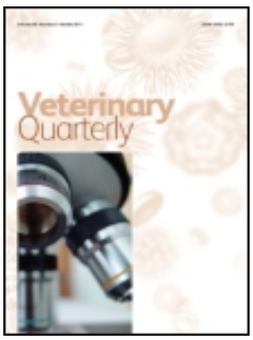

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Biological and pathobiological aspects of the glycocalyx of the small intestinal epithelium. A review

H. J. A. Egberts, J. F. J. G. Koninkx, J. E. van Dijk, and J. M. V. M. Mouwen[1]

SUMMARY *The literature on the glycocalyx of small intestinal epithelium is reviewed. The structure, general and barrier functions, synthesis, and degradation of the glycocalyx, and pathobiological aspects of the glycocalyx in relation to its barrier function are mentioned. Topics for future research are indicated.*

INTRODUCTION

The mucosa of the small intestine is composed of the muscularis mucosae, the lamina propria, and the lamina epithelialis. Roughly, villi and crypts of Lieberkühn can be recognized. The lamina epithelialis consists of a continuous layer of columnar epithelial cells and is separated from the lamina propria by a basal lamina. The epithelium in the crypts is formed by enteroblasts, goblet cells, various kinds of entero-endocrine cells, a few tuft cells and, in most mammalian species, Paneth cells (1). The epithelium covering the villi is mainly composed of enterocytes and goblet cells, and a few entero-endocrine cells, tuft cells, and the recently discovered 'cup' cell (1, 2). At the apical border, the epithelial cells are attached to each other by the tight junctions (3). Between the epithelial cells lymphocytes are situated (4). Solitary and aggregated lymphatic nodules are scattered in the lamina propria and in the submucosa throughout the small intestine. Within the epithelium overlaying this lymphatic tissue, specialized follicle associated epithelial (FAE-) or membranous (M-)cells are present (5-7).

The most important cells with regard to digestion and absorption are the enterocytes. As specializations of their free surfaces they possess microvilli, through which a considerable enlargement of the absorbing surface area is created (8). This 'brush border' is covered by the glycocalyx, a carbohydrate cell coat composed of the oligosaccharide chains of integral plasma membrane glycoproteins and glycolipids (9-11). Being closely connected with the outer world, the epithelium of the gastro-intestinal tract of all animal species has a strategic localization. Nutrients entering the body have to cross this epithelium before they reach the blood or lymph circulation. This uptake of nutrients includes the danger of ingesting injurious material, which can damage and/or cross the intestinal lining. Therefore, in addition to the function of digestion and absorption of nutrients, the intestinal epithelium has also to fulfil the function of a barrier to noxious products. Based upon data from the literature, Mouwen *et al.* (12) proposed the concept of a three component small intestinal mucosal barrier, consisting of the following immunological and non-immunological mucosal defence mechanisms:

- the mucus layer on the mucosal surface, forming the outermost mucosal barrier;
- the epithelial layer with the glycocalyx, tight junctions, lysosomes, and basal lamina;
- the lympho-reticular system of the mucosa, including the gut-associated lymphoid tissue (GALT).

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Present knowledge concerning the mucus layer has recently been reviewed (12). The purpose of the present article is to review some biological and pathobiological aspects of the glycocalyx of the small intestinal epithelium in relation to cellular processes and to its possible role in the small intestinal mucosal barrier.

BIOLOGICAL ASPECTS OF THE GLYCOCALYX

Structural aspects of the glycocalyx

Bennett (13) suggested that every cell might possess a carbohydrate coating on the outer surface of its plasma membrane. He proposed the term glycocalyx. This hypothesis has been confirmed in a large number of rat cell types (14, 15). Ito noticed a conspicuous glycocalyx on intestinal microvilli (8). Over the entire length of the small and large intestine the glycocalyx is seen as a uniform layer of filamentous material (Fig. 1), rarely less than 100 nm and sometimes more than 500 nm in thickness (16, 17). Because the glycocalyx of goblet cells and that of the M-cells is less prominent, compared with the adjacent enterocytes, an abrupt change in thickness of the glycocalyx may be seen at these cells (18). The glycocalyx is equally well developed on enterocytes at the base and near the tip of the villus (18); the filaments have a diameter of 2.5-5 nm and branch freely (17). In the crypts the glycocalyx is less abundant, while the filaments are less firmly packed and show less branching (19). The fact that the filaments are longer and more numerous at the tips of the microvilli than in the clefts between them (15) probably accounts for the more intense staining reactions seen in both light and electron microscopy of the apex of the brush border (17). The glycocalyx appears distinctly different in its fine structure from the mucus, and its filaments appear to be integrated with the cell membrane (16, 17, 20). Therefore, the mucous layer and the glycocalyx are two different structures.

The appearance of the glycocalyx seems to vary with the preparative and staining methods used. Sometimes the glycocalyx is observed as a thick meshwork of branching filaments (16, 21), but in other studies the filaments are arranged in a more parallel way and do not branch (22). In negatively

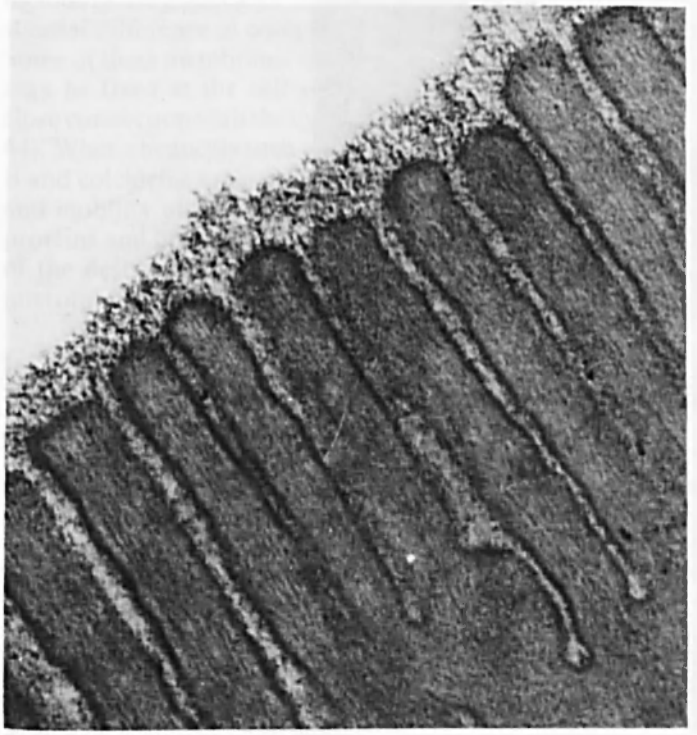

Fig. 1. The glycocalyx covering the microvilli of a rat enterocyte. Uranyl acetate and lead citrate staining (x 150.000).

stained isolated brush borders 600-nm knobs have been seen, which are presumed to represent the glycocalyx (23). In some studies (19) the glycocalyx shows a substantial variation in distribution in the small intestine, in other observations a glycocalyx of fairly uniform thickness is found (16). Even a variation in thickness of the glycocalyx covering individual enterocytes has been described, which has been interpreted as a result of differences in the maturity of the enterocytes (22, 24).

The presence of a glycocalyx does not depend upon taxonomic classification. However, in man, cat, and bat, the glycocalyx appears to be thicker than in some other animal species (9). Glycocalyces have been clearly demonstrated in the intestine of insects (25-27), amphibians (9, 28), and fish (Fig. 2).

As mentioned in the introduction, the glycocalyx is composed of the carbohydrate moieties of the plasma membrane glycoproteins and glycolipids. The enterocyte membrane has been shown to be very rich in these two glycoconjugates (29).

Glycoproteins are polypeptide chains with 1-15, and sometimes even more, sugar units covalently linked to the protein core by a type specific glycosidic linkage. The glycocalyx glycoproteins show a N-glycosidic linkage, whereby the polysaccharide chain is joined by the linkage sugar N-acetylglucosamine via a nitrogen bridge to asparagine in the peptide chain (30, 31). The carbohydrate chains, which can be branched, contain the following monosaccharides: acetylglucosamine, acetylgalactosamine, galactose, fucose, mannose, glucose, and sialic acid (10, 32, 33). The sialic acids are chiefly responsible for the negative charge of the glycoproteins, because they always occupy a terminal position in the carbohydrate chains. Sulphation of sugars also contributes to the acidity of the glycoproteins (9) and therefore to the negative charge of the glycocalyx and plasma membrane (34, 35).

Glycolipids are glycoconjugates with 2-8 monosaccharides linked to a ceramide molecule (36). The carbohydrate chains of the glycolipids resemble those of the glycoproteins of the same tissue, but may sometimes differ in the composition of the central

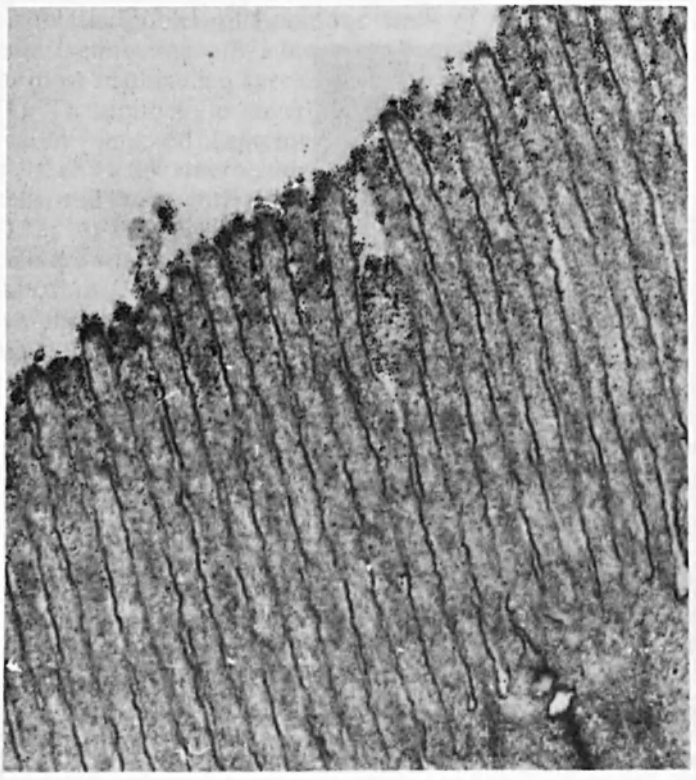

Fig. 2. Labelling with cationized ferritin of the glycocalix of a trout enterocyte (x 70.000).

sugars (33). The external parts of the sugar chains are identical with those of the glycoproteins; hence the polar group of the glycolipid molecule is negatively charged at a physiological pH.

The cell membrane consists of two layers of phospholipid molecules, their hydrophilic, polar groups facing outwards from the membrane. The lipid or protein moiety of the glycoconjugates are thought to be inserted in this membrane matrix, whereas the carbohydrate part protrudes freely into the extracellular space (37). The glycoproteins penetrate the membrane to various depths, depending upon the nature of the protein unit. In the 'fluid-mosaic' model (38) the membrane components are presumed to diffuse in a lateral plane of the membrane. This movement can, however, be limited by several structures, including extracellular mucus, tight junctions, and cytoskeletal elements (37).

The tight junction is situated just beneath the brush border at the apical boundary of adjacent epithelial cells, closely connected with cytoskeletal elements. Because of its property of restricting the lateral diffusion of macromolecules in the membrane, the apical and basolateral plasma membrane, including the glycocalyx, may show a substantial difference in composition (39-41). Some of these membranal macromolecules may be fixed at the cell surface by their close connection with the cytoskeleton (42-44). When chemicals such as cytochalasine B and colchicine are used, the distribution and mobility of the integrated membrane proteins and lipids are influenced, because of the destruction of microfilaments and microtubuli (43).

General function of the glycocalyx

From a vast amount of data in the literature the conclusion is inescapable that the membrane glycoconjugates differ widely in their function. It has become clear that a number of biological functions of the cell are in fact properties of the membrane glycoconjugates (11, 16, 45).

Because of its negative charge, the glycocalyx may function in ion exchange (20) and is able in this way to modify the cationic environment of the cell. Cell membranes treated with neuraminidase reveal a decreased K^+-flow, indicating that sialic acid plays a role in K^+-transport (46). Jarnefelt *et al.* (31) have stated that the position of the terminal branches of the carbohydrate side chain may be responsible for a function specific 'fingerprint'. For instance is was noticed that tissue glycoproteins contained disialosyl sequences in terminal position, while these structures never occurred in circulating glycoproteins (47). Moreover, the glycocalyx of the intestinal epithelial cells contained more mannose units in its oligosaccharide chains than the cells of the respiratory tract did (39). This variability causes the elements of the glycocalyx to function as antigens, such as blood group antigens (42), histocompatibility antigens (48, 49), and carcino-embryonic antigens (50, 51).

The intercellular 'cement substance' between epithelial cells appears to be also a property of the glycocalyx (15). Indeed, it can be postulated that cellular recognition and adherence is a principal function of the glycocalyx. This phenomenon is involved in a number of events, including mitosis, contact inhibition, cellular adhesion, and the subsequent formation of more intricated junctions, a process in which the Ca^{++} plays an important role (52-56).

A specific pattern of sugar moieties in a carbohydrate side chain may also serve as a recognition site for other complementary structures. As a consequence, glycocalyx elements may function as receptors for bacteria, viruses, toxins, lectins, antibodies, interferon, hormones, Vitamine B_{12}-intrinsic factor complex, heme (57-60), and so on.

The microvillous membrane, including the glycocalyx of the intestinal epithelium, contains both hydrolytic enzymes (e.g. disaccharidases, dipeptidases) and synthetic ones (e.g. glycosyltransferases) and possesses the carrier proteins involved in sugar and amino acid transport (61-70). Sialyltransferase activity is greatest in the villous cell surface membrane, but galactosyltransferase is most active in the crypt cell surface, as it is in the foetal intestinal epithelium (71).

Within the framework of this article we have to mention two enzymes separately, because of their function in the intestinal

barrier model; the carbohydrate side chains of these glycoproteins are part of the glycocalyx. The first one is aminopeptidase, which splits peptides into their constituting amino acids and plays a role in the transport of these amino acids. The enzyme is composed of two distinctly different parts, a hydrophilic moiety, which bears the total carbohydrate content together with the enzyme activity, and a transmembranal hydrophobic part, which has been shown to act as a transport unit (68). It may also function as a transmitter of biological information between the inside and the outside of the cell (68). The second brush border enzyme to be mentioned is enteropeptidase, formerly known as enterokinase. It forms the initial activator for the inactive precursors of trypsine, chymotrypsine, carboxypeptidase A and B, and elastase, which are all secreted by the pancreas. Indeed, it has been shown that pancreatic proteolytic enzymes bind to the glycocalyx, indicating that this binding might be a prerequisite to activation by enteropeptidase (72).

Barrier function of the glycocalyx

The glycocalyx is the first cellular structure with which ingested materials come into contact, and from the previous sections of this article the conclusion can be drawn that the glycocalyx must have considerable protective properties. First of all, being of glycoprotein nature, the glycocalyx may behave as a continuation of the mucus layer superimposed on it, so that it may act as a lubricant and sieve to large molecules and micro-organisms (9, 12, 17, 20).

A second barrier is attributable to the sialosylation and/or sulphation of the oligosaccharide chains of the glycocalyx, which protects the membrane proteins from breakdown by pancreatic enzymes (9, 73). Disaccharidases do not have sialic acid bound to their carbohydrate side chains (74). Furthermore, a proteolytic degradation of membranal components occurs when sialic acids are removed from the sialosylated glycoproteins (75). Therefore, normally non-sialosylated brush border components are probably protected from proteolysis by sulphated groups *in vivo*.

Sialosylated components of the glycocalyx in their turn are found to be protected further by O-acetylation (10, 76). As a result, the terminal sugars become resistant to neuraminidases, which may be derived from lysosomes of desquamated cells or produced by several bacteria and viruses. This state hinders the attachment of the micro-organisms to the glycocalyx.

A rapid turn-over of the glycocalyx provides a clearing system for the mucosal surface (77). Ugolev proposed his model for membrane-bound digestion by showing that the glycocalyx is involved in the intestinal barrier (64). In this type of digestion pancreatic enzymes adsorbed to the glycocalyx and intrinsic membrane enzymes are involved. Pancreatic enzymes, secreted by the pancreas as proenzymes, may be adsorbed to the glycocalyx surface, distributed throughout the glycocalyx, and/or after penetration into the glycocalyx, adsorbed to the outer leaflet of the trilaminar membrane. Ugolev *et al.* (78) have been able to separate the apical glycocalyx from the remaining enterocyte membrane, and analysis of these two fractions showed that 60 per cent of the α-amylase and about 80 per cent of the trypsin activities were concentrated in the apical portion of the glycocalyx, suggesting a considerable barrier to suger and peptide polymers. Once these substances have been depolymerized, and diffusion has occurred into the deeper parts of the glycocalyx, they are hydrolyzed by the intrinsic brush border enzymes into absorbable units.

Absorption of non-hydrolyzed molecules by the mammalian small intestine presupposes that such molecules have resisted the digestive capacity of the glycocalyx (72, 79). This can only happen if the amount of ingested dietary molecules is so considerable that many molecules escape hydrolytic destruction, or if the capacity of the glycocalyx enzymes is diminished. Such a surplus is subsequently absorbed by the cell through an endocytotic process, and if a complete lysosomal breakdown also fails, the molecules may be transported to the lamina propria. Another possibility is the paracellular transport of molecules which have passed the glycocalyx. These molecules may exert a direct, toxic, influence on intestinal cells. If the molecules are immun-

ogenic, a local or even general immunological effect may be elicited (80).
Thus, in the case of humoral activity, IgA may be produced. Two IgA molecules are coupled by the J-chain. This IgA dimer is subsequently linked to a secretory component (SC), a product of the enterocyte (81), at the basolateral cell membrane. The SIgA complexes are transported to the glycocalyx through an exocytotic process and eventually become incorporated into the glycocalyx (82). The SC stabilizes the IgA dimer and, being a glycoprotein itself, it renders the complex less susceptible to proteolytic attack (83). The antibodies in the glycocalyx are able to react with the appropriate antigen to form complexes which are unable to bind to the cell membrane. This results in an enhanced breakdown by hydrolytic pancreatic and brush border enzymes (80, 84, 112). The importance of this barrier system is demonstrated by the absorption of dietary antigens in patients with a SIgA deficiency, which leads to the development of high levels of serum antibodes against dietary proteins (82). A small amount of intraluminal protein traverses the gut and reaches the lymphoid organs intact (83), in order to function as a primary antigen or as a constant immunological booster (85). This antigen uptake in the adult is probably the main function of the M-cells (86, 87).

The indigenous mucosal flora of the intestine turns out to be recognized by the host as 'self', because against their antigens no antibodies are produced (88). The practical effect of an indigenous microflora is the exclusion of newly introduced bacteria. Intestinal bacteria retard the local growth of Salmonella and Shigella (82). The glycocalyx provides the indigenous micro-organisms with special attachement 'cups', as demonstrated by Wagner and Barnett (88) and Neutra (89).
A possible model of the barrier provided by the glycocalyx is drawn in Figure 3.

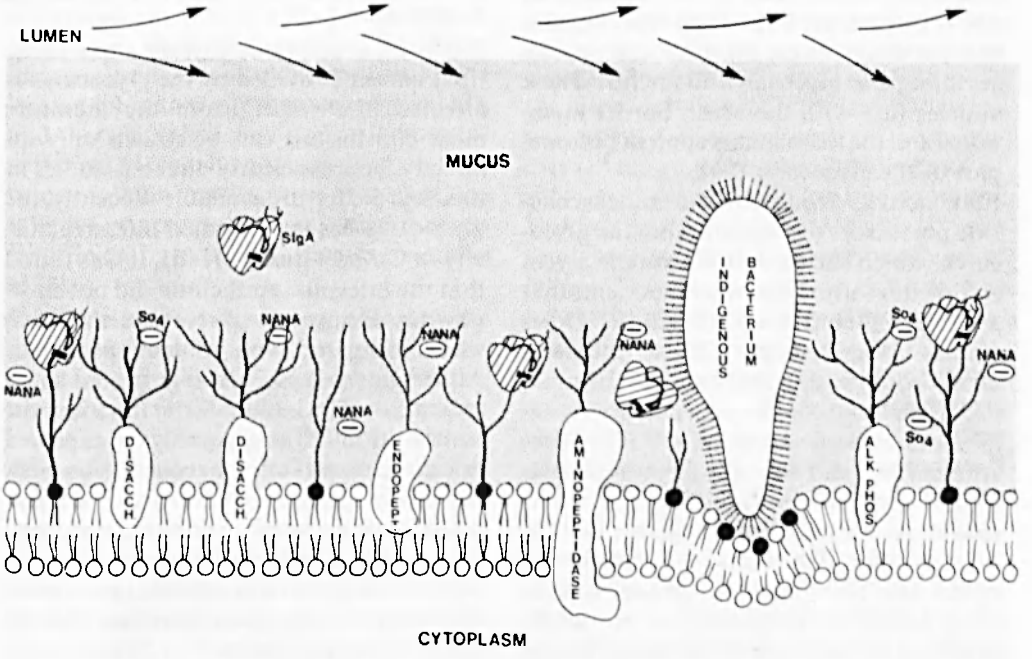

Fig. 3. A schematic representation of the barrier functions provided by the glycocalyx (disacch. = disaccharidase; endopept. = endopeptidase; alk. phos. = alkaline phosphatase; NANA = N-acetyl neuraminic acid; SO_4 = sulphated group).

Synthesis and degradation of the glycocalyx

The formation of the glycocalyx starts with the biosynthesis of the protein and lipid part in the RER and SER, respectively. During migration to and residence in the Golgi system glycosylation occurs. Autoradiographic studies have led to the conclusion that in the RER mannose (90) and glucosamine (90-92) residues are synthesized into a core of sugars, which is connected with the protein part, whereas the uptake of fucose (92-94), galactose (95), and sialic acid or its precursor N-acetyl mannosamine (96, 97) takes place in the Golgi system where the peripheral parts of the side chains are synthesized (90).

The glycosylation of lipids occurs in the SER and Golgi region by the successive addition of monosaccharides (42). The individual monosaccharides are taken from the cytoplasmatic pool and linked to the growing sugar chain by specific glycosyltransferases, after being transformed into nucleotide sugars (42, 98-100). Simultaneously with the disappearance of the radioactivity from the Golgi region, a significant increase in labelling occurs in the apical vesicles which is followed by the labelling of the brush border (92, 101). The vesicles contain filamentous material strongly resembling the glycocalyx filaments. These vesicles fuse with the brush border membrane and the filamentous content becomes part of the glycocalyx (94).

Radioactivity from labelled monosaccharide precursors disappears from the glycocalyx, which shows that membrane glycoconjugates form dynamic components that are renewed constantly (16, 92, 97). Other studies using enzyme activity measurements confirm this renewal (71). Intrinsic intestinal brush border enzymes do not exhibit uniform turn-over rates. For instance Alpers (102) showed that large molecular weight glycoproteins, such as the disaccharidases, turned over at a faster rate than smaller ones. Pancreatic proteolytic enzymes and lysosomal hydrolases released after epithelial desquamation are implicated in the turn-over of the brush border enzymes (103, 104). Elastase in particular might play an important role in this process (104). Also microbial proteases are shown to affect brush border enzyme activity (105). Moreover, the brush border is constantly exposed to the erosive effects of moving chyme (106) and to the degradative action of dietary lectins (107). In feeding experiments, biliary salts such as taurocholate appear to solubilize brush border enzymes (71, 108), and biliary salts combined with elastase cause augmented destruction of the brush border components (71). The turn-over is also achieved by membrane shedding, a process in which small pieces of microvillous membrane are shed into the lumen (109).

The activity of some enzymes located in the glycocalyx varies with a diurnal rythm related to feeding cycles (110-112). The activities of the intestinal disaccharidases are also known to respond adaptively to diet. Feeding a diet with a high carbohydrate content results in higher activities of sucrase and maltase (113). These variations in activity can be explained by a change in enzyme synthesis or degradation rate, a change in enzyme activity, or a combination of these possibilities (114).

PATHOBIOLOGICAL ASPECTS OF THE GLYCOCALYX IN RELATION TO ITS BARRIER FUNCTION

Little is known about how the small intestinal barrier provided by the glycocalyx is affected in disease. From the literature most conclusions can be drawn only indirectly, because clearly directed studies in this field are hardly available. Recently, the glycocalyx has been studied ultrastructurally in Crohn's disease (115). It was found that the intestinal epithelium did not show obvious changes in glycocalyx thickness when compared with control specimens. Although this has been interpreted as an indication of an intact barrier function, the composition of the glycocalyx is expected to be important for its barrier role as well. Generally speaking, inferiority of the glycocalyx barrier may be due to a disturbed synthesis (e.g. inherited defects, deregulation of the protein synthesis, undifferentiated epithelium), or a direct or indirect injury of the glycocalyx.

Inherited defects of the glycocalyx encompass, for example, enzyme deficiencies, resulting in diminished hydrolysis and possibly enhanced uptake of macromolecules.

Endopeptidase (enterokinase) deficiency prevents the activation of several pro-enzymes (116); lactase and iso-maltase-sucrase complex deficiencies have also been described (117, 118).

Inferiority of the glycocalyx as a consequence of a deregulated protein synthesis of the epithelial cell can be caused, for example, by viruses and drugs. After being incorporated by endocytosis, a virus suppresses the host cell's protein synthesis by deregulating the DNA and RNA metabolism of the host, and this may result in insufficient glycocalyx production (119). A decrease in the glycocalyx thickness has been demonstrated in experimental Newcastle disease (120). In the course of the infection the virus uses the cellular mechanisms of the host for the production of its own structural components, followed by the incorporation of the virus envelop glycoproteins in the plasma membrane (121, 122). Hence, before exocytosis of virus occurs, the host cell membrane contains abnormal glycoproteins, which might result in an impaired barrier function. Indeed, incomplete carbohydrate side chains of glycoproteins have been described in virally transformed cells (120). Drugs such as salicylates, phenylbutazone, corticosteroids, tetracyclines, and chloramphenicol are known inhibitors of protein synthesis (12), and therefore they may also induce an insufficient glycocalyx. A selective IgA-deficiency in man results in an increased uptake of macromolecules, suggesting an insufficient glycocalyx barrier function (123).

Furthermore, a disturbed synthesis of the glycocalyx may be associated with a delayed maturing of the intestinal epithelium or epithelial regeneration after the epithelium has been damaged (124). Probably, a delayed maturing of the intestinal epithelium is expressed by the cell membrane composition, in a similar way as the epithelium of immature animals differs from mature ones (125-128). The enhanced permeation of macromolecules in young animals as compared to that in adults, and the increased cholera enterotoxin binding to intestinal membranes of newborns compared to those of adults may be related to differences in the membrane composition of the developing epithelium (125, 129). An inferior glycocalyx can be expected in all cases in which damage of the intestinal epithelium has been followed by regeneration, e.g. in villous atrophy (130). Thus, in tropical sprue or coeliac sprue in man, a deficiency of dissaccharidases and peptide hydrolase activity occurs (131), while also in methotrexate induced villous atrophy in rats the brush border enzyme profile of villous cells resembles that of the crypt cells (132). Incomplete carbohydrate side chains of glycocalyx glycoconjugates have been described in coeliac sprue (107, 133). The inflamed small intestine, accompanied by villous atrophy, appears to be more permeable to macromolecules than the normal small intestine (134, 135), possibly due to an insufficient glycocalyx of immature villous epithelium.

Direct injury of the glycocalyx can be caused by infectious agents (e.g. viruses, bacteria, protozoa) as well as by non-infectious agents (e.g. lectins, bile salts, lysolecithine, cations). In viral infections the sialic acid is removed by the viral neuraminidase, prior to binding of the virus to the carbohydrate side chains of specific receptors (57) An analogous mechanism is seen in bacterial infections. Electron microscopic studies showed that attachment of Vibrio to the intestinal epithelial surface was accompanied by a disappearance of the glycocalyx. This might be due to the removal of sialic acid from the glycoproteins by the Vibrio neuraminidase, which is also thought to potentiate the enterotoxin effect (136, 137). Bacterial pili may play a specific role in the adhesion of *E. coli* to the epithelium of the small intestine (138, 139). Attachment of bacteria takes place only to the enterocytes of the villous, suggesting that the presence of one or more cell surface components of differentiated enterocytes is needed for binding (89). It was found by several workers that these receptors were D-mannose for *Salmonella typhi* and *E. coli* (59, 140, 141) and L-fucose for *Vibrio cholera* (142). The cell receptors for cholera enterotoxin, choleragenoid, and *E. coli* enterotoxin are monosialogangliosides Gm_1 (143). From these data one might conclude that the enterocyte possesses directly accessible attachment sites for pathogenic

microorganisms. In contrast we had rather considered the possibility that in an ideal situation acetylated sialic acids protected hidden recognition sites for viruses and bacteria, and that there had to be an initial deacetylation step, before bacterial or viral neuraminidase was able to split off the sialic acid and binding could occur to the exposed receptors. Whether, and how, such deacetylation takes place in the pathogenesis of these infectious diseases is unknown.
In Giardia infection a significantly impaired barrier function of the glycocalyx is presumed because of the appearance of lower disaccharidase levels, partly due to damage to enterocyte microvilli (144) and we observe a decreased thickness of the glycocalyx (Fig. 4).
Dietary lectins are known to degrade the glycocalyx (107), whereas biliary salts such as taurocholate appear to solubilize brush border enzymes (62. 70), resulting in increased permeability (145). In a rat experimental model, high concentrations of lysolecithine impaired the small-intestinal mucosal barrier and enabled macromolecules to permeate, possibly due to an interaction of lysolecithine with the protein portion of the plasma membrane (146). The polyvalent cation polyornithine causes an increased intestinal permeation of macromolecules in neonatal piglets, possibly as a result of an alteration of the charge of the brush border membrane (147). A direct injury of the glycocalyx by noxious agents is also dependent on their admittance to the cell membrane, which at least in part depends on the quantity and quality of the overlying mucus layer (12).
Lastly, an indirect injury of the glycocalyx may occur as a consequence of cell damage.
In conclusion we propose that in fact each alteration of the glycocalyx, with its specific biological function, may have some effect on the intestinal glycocalyx-derived barrier.

TOPICS FOR FUTURE RESEARCH

Although many features of the small intestinal glycocalyx have been elucidated in the past twenty years, much has still to be learned concerning the (patho)biology of the glycocalyx.

Fig. 4. *Giardia* infection of a rat small intestine. Note the decrease in thickness of the glycocalyx at the attachment site (x 75.000).

Future research ought to concentrate on the following matters:

— the production and composition (sialosylation, O-acetylation of sialic acids, and sulphation) of glycocalyx glycoconjugates in normal and diseased small intestine;
— the correlation between ultrastructural appearance and functional integrity of the glycocalyx in the normal and the diseased small intestine;
— the effect of drugs or infectious agents on glycocalyx composition and function.

To answer these questions we suggest *in vivo* studies in animal models. A combination of ultrastructural, histochemical, and biochemical methods may provide us with a better understanding of this cellular structure.

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